PB-217 487

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Public Health Service
Cincinnati, Ohio

1935

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DETERMINING THE BACTERIOLOGICAL QUALITY OF DRINKING WATER.*

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Although it has long been known that pathogenic bacteria in water have been the causative agents in innumerable epidemics of intestinal diseases such as typhoid fever, cholera and dysentery, there is as yet no satisfactory procedure available to the average laboratory for determining promptly and definitely the freedom of any water supply from these pathogens. However, because of their natural habitat, intestinal pathogenic bacteria are never found in nature, except in combination with other classes of bacteria that abound in the normal feces of man and warm blooded animals. Of these, Bact. coli are always present in enormous numbers and Bact. aerogenes to a lesser extent. Although it is definitely proven that bacteria of this latter group also occur on certain vegetation and in virgin soil, nevertheless, these two genera have very many characteristics in common. For these reasons they usually have been grouped together and first were designated as the B.coli group and later the coli-aerogenes group. Thus it is that although bacteria of the coli-aerogenes group, insofar as we know, do not cause disease and are not a factor in our well being, their presence in a water, because of their origin, has been recognized as an indicator that such water

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(*) For Presentation, Annual Convention American Water Works Association, Cincinnati, Ohio, May 8-10, 1935.

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is polluted with sewage or surface drainage, or both, and hence may also contain intestinal pathogens. Furthermore, the concentration of coli-aerogenes group organisms in water has been considered as a measure of the extent of the pollution which has occurred.

It is upon this basis of coli-aerogenes density that accepted bacteriological standards for the safety of water for drinking and culinary purposes rests. The bacteriological section of the Treasury Department standard is of this nature and includes both sections of the coli-aerogenes group determined under definite specified procedures. Recently much has been written about the elimination of the aerogenes group from consideration in this connection on the assumption that this genus if present in the water may have originated from sources other than the human body. Restrictive media, differentiation procedures and various other devices have been suggested for selective development of the Bact.coli section and suppression of the aerogenes section of the bacteria composing the coli-aerogenes group in water. It need only be remarked that any narrowing of this group by any of these methods, regardless of their accuracy or practicability, has the ultimate, practical effect of lowering the established limiting standard, that is of increasing the permissible density of coli-aerogenes organisms. Therefore, if such analytical procedures are practiced, this standard limit should be tightened accordingly if we are to retain our present degree of purity of drinking water supplies.

The present accepted procedure for determining the presence and density of organisms of the coli-aerogenes group in water is the dilution method using a liquid media. For the examination of drinking waters to determine their conformity to the Treasury Department standard, 5 - 10 cc. portions of the sample are inoculated into tubes of standard broth and the cultures producing gas further confirmed for definite characteristics of the group. The limit of organisms permitted under this determination is 1.05 per 100 cc. of water. Inasmuch as the 5 - 10 cc. tubes thus inoculated will measure not less than 2.2 organisms per cc., the method is too coarse for ascertaining accurately the coli density within the desired range. This deficiency can be overcome, in part at least, as has been pointed out before(1), by examining larger size portions of sample, say 50 cc. or 100 cc.

This point is clearly illustrated by the results, kindly furnished through the courtesy of Mr. Harry E. Jordan of the Indianapolis plant for the year 1934, where both 5 - 100 cc. and 5 - 10 cc. portions of the finished water are examined. During the entire year, out of 365 daily samples, on only two days did the 10 cc. portions indicate the presence of members of the coli-aerogenes group, while 19 - 100 cc. portions were positive. Moreover, in one month during seven different days, a total of 9 - 100 cc. portions were positive, indicating that some abnormal circumstance was permitting a slight yet discernible deterioration of the high bacteriological quality, but which was altogether undetectable by the 10 cc. portion analyses.

Again our examination of Cincinnati tap samples over a period

of about a month disclosed a total of 21 - 100 cc. portions out of 165 positive for coli-aerogenes, whereas during the same period only 5 - 10 cc. portions were confirmed. The results in this case also show a continued day to day appearance of positive 100 cc. tubes indicating the approach of higher bacterial numbers not so clearly disclosed by the results from the 10 cc. portions examined, although the bacterial density was evidently frequently sufficient to be reflected in the 10 cc. portions of the samples.

Interpretation of Analytical Results.

The Phelps' index or reciprocal method of interpretation of the analytical result from the liquid media determination as given in the present Standard Methods does not apply to the case of a multiple number of tube results at only one dilution or in fact to more than one tube in each of a series of dilutions. Even in the single tube dilution series for which it was designed, the principle is not mathematically sound. Moreover, the present edition of Standard Methods contains no suggestion for the interpretation of multiple tube analyses. There is definite need, therefore, when the multiple tube procedure is employed, as it now generally is, for some uniform method of properly interpreting such analytical results and of expressing them in comparable terms of bacterial density per unit volume of sample, regardless of the number and size of portions planted at one or more dilutions. The most generally accepted procedure for this purpose is that of the Most Probable Number (M.P.N.). The Most Probable Number in simple

language is nothing more than that bacterial density, which, if it had been actually present in the sample under examination, would, more frequently than any other, have given the observed analytical result.

The theory and method of computation of the M.P.N. has been developed by various writers over a considerable period of years in this country and abroad. Simplification of the somewhat involved mathematical treatment has been suggested by one of us[2] and tables of M.P.N. values corresponding to the combinations of portions of sample most commonly planted in routine analytical work have been made available[3][4][1].

Where only equal portions, at a single dilution are planted, the result may be interpreted in terms of the M.P.N. value, as Greenwood and Yule[5] state, by the formula:

$$N\lambda = 2.302585 \log \frac{K}{q}$$

Where N = size in cc. of the portion examined,

K = number of portions of size N,

q = number of portions showing no growth,

λ = M.P.N. per cc. of sample.

This expression can readily be plotted in the form of a curve, (Fig. 1), from which may be read direct the M.P.N. corresponding to any observed analytical result where any number of tubes have been inoculated with the same size portion of the sample.

M.P.N. values corresponding to results from more than one size portion are more easily obtained from tabulated computations of such values as mentioned above or, if necessary, by computation direct. However, an approximate value for such a series of dilutions may be obtained by employing the above formula or the chart, using for the purpose the results from the middle dilution of the series.

The optimum size of inoculum.

It is always advisable in planting portions of a sample in nutrient media tubes to select that size of portion which will give a determinative result, that is, one in which not all tubes show no growth or all show growth. The optimum size portion obviously would be such that equal numbers of tubes show growth and no growth, or, expressed in another way, in an extensive series of multiple tube tests of the same size portion of sample the smallest possible percentage of tests should be indeterminate. In other words the sum of these all positive and all negative tests should be a minimum.

An examination of the expansion of the binomial giving the frequencies of occurrence of the different results which will be obtained in the analysis of the sample, discloses that the optimum size of portion to be planted, when equal portions are employed, is given by the expression

$$e^{-M\lambda} = 0.5 = e^{-0.693}$$

$$\text{or} \quad M\lambda = 0.693 \quad \text{or} \quad M = \frac{0.693}{\lambda} \qquad (1)$$

where N = size of portion in cc.

λ = M.P.N. per cc.

Hence, if the approximate bacterial content (λ) of the sample to be examined can be estimated, the size of portion to plant (N) can be readily obtained from this relationship or by interpolation from the chart (Fig. 2) which is a graph of this equation (1). This same procedure or chart may be used to ascertain the optimum size of the middle portion when different sized portions in geometrical or other series are to be planted.

In any extensive series of multiple tube tests in which the same size portion of sample is employed, the chances are that a definite percentage of the tests will produce all positive or all negative results, the exact number being a function of the concentration of coli-aerogenes organisms, and size and number of portions examined. All such results are, of course, indeterminate for the M.P.N. value. In general, when equal portions of the sample are planted, the largest percentage of such series that will give a determinate result (some positive and some negative tubes) is given by the relationship:

$$P = 100 \left[1 - (e^{-N\lambda})^K - (1 - e^{-N\lambda})^K \right] \qquad (2)$$

when P = the percentage of samples that will give a determinate result,

N = size in cc. of portion of sample examined,

λ = M.P.N. per cc.

K = number of portions of size N in each test.

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If now the optimum size portion N as determined in (1) is used, then $e^{-N\lambda} = .5$ and substituting this value in (2)

$$P = 100\left[1 - 2(.5)^K\right] \qquad (3)$$

Employing these formulae, it is possible to compute the percentage of determinate results that will be obtained in any large series of tests when any number of tubes are used in each test. In Table 1, are presented computed values of P when the optimum portion of sample is planted in any number of tubes from 2 to 10 and also when 1, 10, or 100 cc. portions are planted of a sample containing 1.05 coli-aerogenes group organisms per 100 cc. (the Treasury Department limit).

Table 1. Percentage of determinative results to be expected, P, when a definite number of portions, K, of a sample are planted.

No. of equal size portions planted = K	$N\lambda$ (= size of portion in cc. x concentration per cc.)			
	0.693 (= optimum)	N = 1 λ = 0.0105	N = 10 λ = 0.0105	N = 100 λ = 0.0105
2	50	2.1	19.9	45.5
3	75	3.1	27.0	68.1
4	87.50	4.1	34.3	80.6
5	93.75	5.1	40.8	87.9
6	97.88	6.1	46.7	92.3
7	98.44	7.1	52.1	95.0
8	99.22	8.1	56.8	96.8
9	99.61	9.0	61.1	97.9
10	99.80	10.0	65.0	98.6

An inspection of these data shows, for example, that when only two equal portions of the optimum size are planted, 50 per cent of such series will be either both positive or both negative and hence, not determinative for the M.P.N. value, whereas if 5 such equal portions are planted, 93.75 per cent of the results will be determinative. These data also show clearly the desirability of planting larger portions than 10 cc. amounts when waters approximating in coli-aerogenes content the Treasury Standard for drinking waters are under examination. For example, when 5 - 10 cc. portions of such a water are planted in broth tubes, only 40.8 per cent of such tests would be expected to be determinative (that is 59.2 per cent would either all show growth or all show no growth), whereas if 5 - 100 cc. portions of the same water were used, 87.9 per cent would be determinative (some positive and some negative growth).

For a discussion of the accuracy of the computed M.P.N. result, the reader is referred to discussions of other writers such as Greenwood and Yule[5], Reed[6], Halvorson and Ziegler[7], Allen[8], etc. In general, it may be said that the probable error is large (as much as 150 per cent of the M.P.N.) when a moderate number, say 5 or 10 tubes, is examined. This error is least, however, when from 70 to 80 per cent of the total tubes in a test show growth, depending upon the number of tubes used. Obviously, therefore, in selecting the size of portion to examine as determined by (1) above it would be advisable, for the sake of higher accuracy of the result, to select a size of 1 somewhat larger than the computed value, rather than somewhat smaller, in order that there may result more positive tubes than negative

ones. In any event it seems reasonable to conclude that the dilution tube method cannot result in an accurate enumeration of coli-aerogenes organisms and that, therefore, the need for an acceptable and dependable method still remains.

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